# PATENT COOPERATION TREATY



From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
MICHAEL L. GOLDMAN
NIXON PEABODY LLP
CLINTON SQUARE, P.O. BOX 31051
ROCHESTER, NY 14603-1051

## WRITTEN OPINION

**(PCT Rule 66)**

| | |
|---|---|
| Date of Mailing *(day/month/year)* | 24 FEB 2003 |
| Applicant's or agent's file reference<br>200701/1093 | REPLY DUE<br>within 1 months/days from the above date of mailing |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US01/50857 | 25 October 2001 (25.10.2001) | 27 October 2000 (27.10.2000) |

International Patent Classification (IPC) or both national classification and IPC

IPC(7): C12Q 1/68 and US Cl.: 435/6

Applicant

ADVION BIOSCIENCES, INC.

1. This written opinion is the first (first, etc,) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2 (a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☐ Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

**When?** See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension. See rule 66.2(d).~~

**How?** By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

**Also** For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6

**If no reply is filed,** the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 27 February 2003 (27.02.2003) .

| | |
|---|---|
| Name and mailing address of the IPEA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703)305-3230 | Authorized officer<br>Kathleen M Kerr<br>Telephone No. (703) 308-0196 |

# WRITTEN OPINION

International application No.
PCT/US01/50857

## I. Basis of the opinion

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☒ the description:
pages 1-57, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the claims:
pages 58-75, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the drawings:
pages 1-22, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the sequence listing part of the description:
pages 1-8, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

☒ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed."*

**WRITTEN OPINION**

International application No.

PCT/US01/50857

## VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Claims 6 and 49 are objected to under PCT Rule 66.2(a)(iii) as containing the following defect(s) in the form or contents thereof: the "and" in line 2 should be an ---or---.

**WRITTEN OPINION**

International application No.
PCT/US01/50857

**V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. STATEMENT

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-43, 51, 53-62, 64, 71, and 74-86 | YES |
| | Claims | 44-50, 52, 63, 65-70, 72, 73, and 87 | NO |
| Inventive Step (IS) | Claims | 1-43, 51, 53-62, 64, 71, and 74-86 | YES |
| | Claims | 44-50, 52, 63, 65-70, 72, 73, and 87 | NO |
| Industrial Applicability (IA) | Claims | 1-87 | YES |
| | Claims | NONE | NO |

2. CITATIONS AND EXPLANATIONS
Please See Continuation Sheet

**Supplemental Box**
(To be used when the space in any of the preceding boxes is not sufficient)

TIME LIMIT:
The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

**V. 2. Citations and Explanations:**
Claims 1-43 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest the claimed methods. The closest prior art is described below as applied to anticipate some of claims 44-87. Claims 1 and 23 are the two independent claims in this grouping. Both Claims 1 and 23 require a step of "determining the amounts of each type of the nucleotide analogs in the extension solution after said extending"; this step governs the novelty of the claimed invention since the closest prior art uses mass spectrometry to determine the structure of the extended primers, not the solution remaining after extension. Moreover, this idea of measuring the remaining solution from extension primer steps in SNP identification is not taught or obviated in the prior art. The difference between Claims 1 and 23 is in the amplification step wherein Claim 1 uses a phosphatase and Claim 23 uses a molecular weight filter, both of which are common techniques in the art of SNP detection.

Claims 51, 53-62, 64, 71, and 74-86 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest the claimed methods. The modification of the methods of Claim 44, namely Claims 51 and 71 using λ-exonuclease, is not common in the prior art. The specifics of the methods of Claim 44 with respect to the MALDI-TOF apparatus, Claims 53-62 and 64 and Claims 74-86, are not specifically disclosed in the art

Claims 44-50, 52, and 65 lack an inventive step under PCT Article 33(3) as being obvious over HAFF *et al.* in view of KRAHMER *et al.*
HAFF *et al.* teach detection of SNPs using MALDI-TOF. Specially, HAFF *et al.* teach annealing a primer immediately upstream of the polymorphism on a target nucleotide sequence (double or single-stranded DNA) and using PCR (64°C annealing temperature) to amplify the target nucleotide sequence in the presence of dideoxynucleotides. The amplified sequence PCR mixture is then subjected to shrimp alkaline phosphatase to remove all unreacted dideoxynucleotides followed by heat inactivation of the phosphatase. The amplified target molecule is then subjected to primer extension PCR (37°C annealing temperature) using SNP primers, DNA polymerase, and dideoxynucleotides wherein a single dideoxynucleotide is "extended" onto the amplified target molecule - ddA, ddG, ddC, or ddT depending on the sequence of the SNP. The difference between extending with any one of the four nucleotides is then quantitated via MALDI-TOF.
HAFF *et al.* do not teach using electrospray mass spectrometry for this quantitation step as required in the claims. HAFF *et al.* do not teach using RNA as the sample nucleotide sequence; however, this is an obvious variation that is well known in the art.
KRAHMER *et al.* teach electrospray ionization (ESI) MS and tandem with mass spectrometry for analyzing SNPs.
HAFF *et al.* and KRAHMER *et al.* do not teach treating the amplification primers with exonuclease I follows by inactivation of the enzyme. Such an added purification step is obvious in view of the art.

**Supplemental Box**
(To be used when the space in any of the preceding boxes is not sufficient)

It would have been obvious to one of ordinary skill in the art to combine the teachings of HAFF *et al.* and KRAHMER *et al.* to analyze the results of the experiments of HAFF *et al.* using the easier techniques of KRAHMER *et al.*, specifically because KRAHMER *et al.* peforms nearly the same experiments.

Claims 63, 66-70, 72, 73, and 87 lack an inventive step under PCT Article 33(3) as being obvious over HAFF *et al.* in view of KRAHMER *et al.* and FEI *et al.*
HAFF *et al.* and KRAHMER *et al.* teach as described above. HAFF *et al.* and KRAHMER *et al.* do not teach submitting the PCR product to low molecular weight filtration. Such an added purification step is obvious in view of the art, particularly in FEI *et al.*

Claims 1-87 meet the criteria for industrial applicability as defined by PCT Article 33(4). The detection of SNPs is useful in the detection of inherited diseases such as sickle cell anemia and breast cancer.

---------- NEW CITATIONS ----------

KRAHMER et al. MS for identification of single nucleotide polymorphisms and MS/MS for discrimination of isomeric PCR products. Anal. Chem. Sept 2000, Vol 72, pages 4033-4040.

FEI et al. Analysis of single nucleotide polymorphisms by primer extension and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Communications in Mass Spectrometry. 2000, Vol 14, pages 950-959.